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PROPRIETARY

Date Out EFB: DEC. 16 1980

To: Product Manager 21 - H. Jacoby
TS-767

From: Dr. Willa Garner (S/ Creeger (Acting Chief))
Chief, Review Section No. 1
Environmental Fate Branch

Attached please find the environmental fate review of:

Reg./File No.: 7969-LG

Chemical: Vinclozolin

Type Product: F

Product Name: Ronilan

Company Name: BASF

Submission Purpose: Data Review

ZBB Code: Other

Date in: 10/28/80

Date Completed: DEC. 16 1980

Deferrals To:

_____ Ecological Effects Branch

_____ Residue Chemistry Branch

_____ Toxicology Branch

ACTION CODE: 111

EFB # 659

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TAIS 61

1.0 INTRODUCTION

1.1 Purpose

BASF Wyandotte Corporation submitted additional data in support of registration of Vinclozolin (Ronilan 50W), a new fungicide, intended for use on strawberry [File No. 7969-LG; submitted on 10/2/80]. A comprehensive review of the Environmental Fate of Vinclozolin was completed on 3/25/80. According to this review, registration on strawberry was not acceptable because of some environmental chemistry data gaps in the following studies:

(a) Photolysis.
(b) Soil metabolism study.
(c) Effects of vinclozolin on microbes.

According to EFB review of 9/23/80, the rotational crop data, permits rotation to the following crops:

(a) Leafy vegetables, 6 months after treatment that does not exceed 12 lbs. ai/A.

(b) Cucurbits, 2 months after treatment that does not exceed 9 lbs ai/A.

(c) Corn, 2 months after treatment that does not exceed 9 lbs ai/A provided only the corn grain is used for food and/or feed purposes.

(d) Other grain crops, 9 months after treatment that does not exceed 8 lbs ai/A.

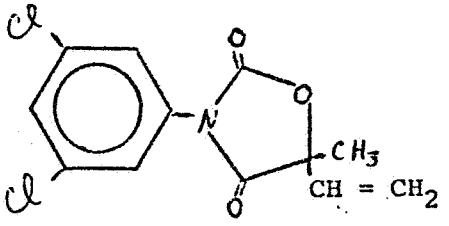
1.2 Chemical

Common Name: Vinclozolin

Trade Name: Ronilan 50W, BAS 352F

Chemical Name: 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione-50WP

Chemical Formula:



Chemical and Physical Properties: See EFB review of 3/25/80.

1.3 Previous Reviews

7969-LG, 9F2205	3/25/80
7969-LG	9/23/80
7969-LG	12/16/80

2.0 USE DIRECTIONS

See EFB review of 3/25/80

3.0 DISCUSSION OF DATA

BASF Wyandotte Corporation submitted additional data as requested in EFB review of 3/25/80. Data were filed under Accession No. 243393, Registration No. 7969-53, dated 12/2/80. The title of each study and data discussion are shown below:

3.1 Photolysis Study

The company submitted additional data to augment the photolysis study previously reviewed, in an attempt to elucidate the fate of vinclozolin in aqueous solution; the light source used; and a profile of the soil used in the soil surface photolysis study. The following studies were submitted:

(a) Stability of Vinclozolin in Aqueous Solution

Ten ml solution containing 5 mg vinclozolin per ml methanol was injected into 10 ml of each buffer medium of various pH ranging from 0 to 5 at final concentration equivalent to 5 mg/kg of vinclozolin.

After periods of 0, 2, 5, 14 and 23 days, one ml samples were drawn from each lot and partitioned with 5 ml hexane. The organic layers were analyzed by GC.

Test results showed that percent recovery of Vinclozolin varied according to pH and time elapsed from start to analysis. It was apparent, however, that Vinclozolin showed the greatest stability at pH 2 where percentage recovery was near 100% and was independent of sampling time.

(b) Unsensitized Photolysis of Vinclozolin

Three mg vinclozolin dissolved in 0.6 ml methanol, was added to a solution of 1.74g H_2SO_4 in 1.5 H_2O in a photoreactor while bubbling air through the mixture giving a final concentration equivalent to 2 mg/kg of an acidic solution (pH 1.94). A mercury lamp TQ 150 was mounted and the solution was irradiated (>280 nm) for a period of up to 1106 hours (46 days).

Two ml samples were drawn at certain irradiation periods and partitioned with 5 ml hexane and the organic layers analyzed by GC.

Test results showed that vinclozolin was stable in aqueous solution at pH ≤ 2. [redacted]. Vinclozolin at concentration 2 mg/kg in acidic aqueous solution (pH 1.94) is not susceptible to unsensitized photolysis by artificial sunlight of wavelengths > 280 nm within 46 days.

(c) Soil Profile Used in the Soil Surface Photolysis Study

As requested in EFB review of 3/25/80, BASF company submitted a profile of the soil used in the soil surface photolysis study. These are: Loamy sand soil, OM 2.5%, pH 6.8, CEC 10 m Val/100g, bulk density 1.4 g/ml, sand 2000-200M 67%, sand 200-20 M 16%, silt 20-2 M 7%, and clay < 2M 10%.

3.2 Soil Metabolism Study

MRID no. 414969-03

BASF Wyandotte submitted additional soil metabolism data as requested in the EFB review of 3/25/80 which are: "the ratio of soil bacteria to fungi to actinomycetes in the European soils and the ratios of soil bacteria to fungi to actinomycetes in American soils common to the strawberry growing areas."

In this study 12 U.S. and one German (standard) soil samples were employed. Table 1 lists soil location, texture, and other characteristics. The objective of this study was to determine the microflora, their frequencies, and Gram+ to Gram-bacteria. Sterile Ringer's solution was used to make dilution series of up to 10^{-6}. Five, 1-ml samples of each dilution series were placed in sterile Petri dishes containing nutrient agar. After incubation for 5-10 days, individual colonies in each dish were counted using a counter.

Test results showed that the ratios of the bacteria/actinomycetes/fungi populations in all the tested soils, fluctuated widely where the German soils lie within the range found for the U.S. soil (see Table 2). The bacteria counts range from 2.04×10^6 to 2.4×10^7. The percentage of Gram-bacteria among the total bacteria ranges from 3.6-15.2%. In the cases of Actinomycetes and fungi, the separate means fluctuate from 5.79×10^5 to 2.81×10^6 means and from 3.15×10^4 to 2.77×10^5, respectively. The German soil had counts of 7.42×10^6 bacteria, 1.55×10^6 Actinomycetes, and 1.47×10^5 fungi, placing it within the given ranges.

It was concluded by the researchers that soil microflora populations were found to be independent of soil texture and soil location.

Table 1: Characterization of the soils

Soil No.	Soil origin	Soil type	Elutable particles % <20 µ	pH KCl	Bulk density g/l	Organic C	Particles % coarse 2000-200 µm	fine 200-20 µm	v.fine 20-2 µm	clay <2 µm	Humus %	Total N %	C/N	EC* meq Ba/100g soil
1	New Jersey	l S	17	4.9	1200	0.99	70	13	5	12	1.7	0.078	13	6.0
2	California	L	39	6.4	1120	1.28	9	52	13	26	2.2	0.128	10	20.0
3	California	c L	40	6.4	1180	1.16	6	54	16	24	2.0	0.126	9	20.0
4	California	L	38	6.4	1150	1.22	13	49	14	24	2.1	0.119	10	19.6
5	New Jersey	l S	15	5.1	1220	1.10	73	12	8	7	1.9	0.078	14	6.0
6	California	c L	43	6.5	1130	1.22	16	41	22	21	2.1	0.111	11	20.0
7	New Jersey	l S	18	5.2	1210	0.81	73	9	12	6	1.4	0.078	10	6.0
8	Florida	S	8	5.3	1410	0.93	85	7	6	2	1.6	0.051	18	6.4
9	Florida	S	8	5.6	1410	0.76	82	10	4	4	1.3	0.031	25	6.4
10	New Jersey	l S	18	4.8	1210	0.99	70	12	12	6	1.7	0.083	12	6.4
11	Michigan	l S	15	5.8	1370	0.76	66	19	9	6	1.3	0.054	14	7.0
12	Michigan	l S	15	6.2	1360	0.64	66	19	11	4	1.1	0.054	12	7.0
13	Neuhofen	l S	15	6.0	1400	2.44	44	41.6	10	5	4.1	0.171	14	12.7

l S = loamy sand; L = loam; c L = clay loam; S = sand

* cation exchange capacity

Table 2. Ratio of bacteria/actinomycetes/fungi in some reprsentative soils.

Soil No.*	Ratio Bacteria	Actinomycetes	Fungi
1	34	9	1
5	28	8	1
7	56	9	1
10	35	5	1
11	155	21	1
12	215	66	1
13	50	11	1

* Same Nos. as in Table 1.

3.3. Effects of vinclozolin on Microbes

BASF Wyandotte submitted additional data as requested in EFB review of 3/25/80 which are: "Effects on N_2-fixation, effects on nitrification, and effects on various enzyme activities of soil microflora." The following studies were submitted:

(a) Influence of Vinclozolin on the N_2-fixation by Azotobacter Chroococcum

In this study, ethylene formation was measured throughout the test period and was used as an index to the nitrogenase activity responsible for N_2-fixation.

In this test, 100 ml nutrient agar was inoculated with 1 ml of Azotobacter suspension under sterile conditions and then incubated in a shaking incubator at 25°C. When cell density reached 5 x 10^6 cells, one ml methanolic solution containing either 1 mg or 5 mg of vinclozolin were added to the test batches. Final concentrations were 10 mg or 50 mg vinclozolin/l of nutrient solution.

Throughout the test, microbial activities, vinclozolin concentration and ethylene production were monitored for a period of 185 hours. Determination of nitrogenase activity was accomplished by using 10 ml sample solution, added to it 2 ml acetylene and shaken for one hour in a shaking incubator at 25°C. The enzyme activity was stopped by adding 0.5 ml of a 30% TCA and the proportion of ethylene formed in the gas mixture was determined using GC. The amount of ethylene liberated from tests and controls were determined in nMol/nour/10^9 cells.

Test results showed that the formation of ethylene gas during the test period from 0-185 hours was not affected by the addition of 10 mg or 50 mg of vinclozolin/l. The researchers concluded that the nitrogenase system which is responsible for the fixation of molecular nitrogen, had not been affected by vinclozolin.

(b) Influence of vinclozolin on nitrification

A loamy sand soil [OM 2.44%; pH 6.0; CEC 10 meq/100g; bulk weight 1.4 g/ml; particle size < 0.002 mm 4.9%, 0.002-0.02 mm 10%, 0.02-0.2 mm 41.6%, and > 0.2 mm 43.5%], was used in this study. One and five mg of vinclozolin, each was dissolved in 1 ml methanol then added to 85 g of soil thus giving an application rates equivalent to 3 kg/ha and 15 kg/ha, respectively. After the solvent had vaporized, 25 mg NH3-N as $(NH_4)_2SO_4$ dissolved in 15 ml H_2O were added. This simultaneously adjusted the soil moisture to 40% of the maximum water capacity of the soil.

Control experiment was run, where nitrapyrin was used as an inhibitor of nitrification. In another control experiment, only the required quantity of $(NH_4)_2\ SO_4$ was added to determine the normal nitrification level.

Samples were taken over a period of 8 weeks for determination of NH_3-N and NO_3-N in the soil. Soil samples were extracted with 1% KAl (SO_4) solution. Portions of the filtered extracts were used to determine NO_3 and NH_3 using NO_3-electrode and titration curves, respectively.

Test results showed that vinclozolin at 10mg/kg soil, shifted the time required to transform 50% of the applied NH_3-N to NO_3-N (t_{50}), from 16 days in non-treated controls to 25 days. A concentration of 50 mg active ingredient/kg soil extended the t_{50} value by 5 more days, to 30 days. In control trials where nitrapyrin was used, the nitrification process was completely inhibited during the 42 days of sampling.

Table 3 shows the transformation values from NH_3-N to NO_3-N in mg/100 gm soil in treated and control trials.

Table 3. Transformation values of NH_3-N to NO_3-N in treated and non-treated soils.

Time Days	mg/100 gm soil							
	Non-treated		10 mg vinclozolin		50 mg vinclozolin		Nitrapyrin	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
7	27	5	23	3	22	3	23	3
14	18	10	24	4	25	4	27	3
21	9	22	19	23	22	11	26	4
28	4	24	11	17	11	12	26	3
35	-	27	9	21	11	21	25	4
42	-	26	2	24	4	24	28	3

(c) Influence of vinclozolin on various enzyme activities of soil microflora

The following enzyme activities were investigated:

1. Amylase
2. Cellulose
3. Dehydrogenase
4. Pectinase
5. Phosphatase
6. Proteinase

1. Amylase Activity

Amylase (0.1%) and glucose (0.2%) were added under sterile conditions to a sterile nutrient salt solution. Solutions were then inoculated with these organisms: Bacillus subtilis, Botrytis cinerea, and Streptomyces aurefaciens. Fourteen days after the experiment was begun, vinclozolin was added to the individual batches of concentration equivalent to 10 mg/l of nutrient solution. Samples were taken at various intervals for determination of enzyme activity by determining the extinction of the solution [U/L], using Zeiss photometer, where "U" is the enzyme activity that converts 1 uMol substrate in 1 min time. Results are shown in Table 4.

Table 4. Amylase Results;

Organism	ai mg/l	Enzyme activity (u/l) after x days: 0	1	3	7	10	14	17	21
Bacillus subtilis	0	124	118	98	94	174	95	93	73
	10	120	118	56	133	133	120	113	81
Botrytis cinerea	0	39	47	23	66	98	20	63	55
	10	43	45	28	104	110	162	67	74
Streptomyces aureofaciens	0	1.0	1.0	0.5	0.5	1.1	1.7	1.5	1.7
	10	1.2	2.2	0.4	1.1	1.3	1.1	1.5	1.4

The researchers concluded that vinclozolin did not affect amylase activity.

2. Cellulase Activity

Glucose (1%), cellulose (0.2%), and ascorbic acid (2.8×10^{-3} Mol), were added to a sterile nutrient salt solution. Solutions were then inoculated with: Bacillus subtilis, Botrytis cinerea, and Streptomyces aureofaciens. Eight days after the experiment was begun, vinclozolin was added to the individual batches at concentrations equivalent to 10 mg/l of nutrient solution. Samples were taken at various intervals for determination of enzyme activity (u/l) using Zeiss photometer. Results are shown in Table 5.

Table 5. Cellulase Results

Organism	ai mg/l	Enzyme activity (u/l) after x days: 0	8	16	23
Bacillus subtilis	0	35	47	81	116
	10	63	127	131	166
Botrytis cinerea	0	8	23	23	22
	10	48	23	22	31
Streptomyces aureofaciens	0	17	23	56	58
	10	0	8	31	31

The researchers concluded that vinclozolin did not affect cellulase activity.

3. Dehydrogenase Activity

Quantities of 120 g H_2O and 8 mg methanolic vinclozolin solution were added to 680 g air-dried sandy soil. Soil moisture was adjusted to 40% and the batches were placed in an incubator at 25°C. Three 10-g samples were taken from each batch at various intervals, and then 3.5 ml nutrient solution that contained glucose (0.2%) and peptone (0.1%)

were added. This was mixed thoroughly and incubated 24 hours at 25°C. Samples were taken at various intervals for determination of enzyme activity, by determining the extension of the solution (in mg H), using Zeiss photometer, where the quantity of H was an index to dehydrogenase activity. Results are shown in Table 6.

Table 6. Dehydrogenase Results

Enzyme activity (H/kg soil) after x days

ai mg/l	0	7	14	21	28
0	430	510	180	250	340
10	510	500	190	180	330

The researchers concluded that vinclozolin did not affect dehydrogenase activity.

4. Pectinase Activity

Glucose (1%) and pectin (0.1%) were added to sterile synthetic nutrient solution. Solutions were then inoculated with: Bacillus polymyxa, Fusarium oxysporum Lycopersici, and Streptomyces aureofaciens. Seven days after beginning the experiment, vinclozolin was added at a final concentration equivalent to 10 mg/l solution. Samples were taken at various intervals and enzyme activity was determined by adding polygalacturonic acid and measuring solution viscosity after 48 hours incubation. Results are shown in Table 7.

Table 7. Pectinase Results

		n(Δ Pa. sec) decrease in viscosity			
Organism	ai mg/l	0	7	14	21
Bacillus	0	1.5	0.4	0.5	0.7
polymyxa	10	0.9	1.1	0.2	0.6
Fusarium	0	0.7	0.2	1.4	0.4
oxysporum	10	0.8	0.3	1.2	0.8
Streptomyces	0	1.2	0.3	0.8	0.2
aureofaciens	10	0.9	0.2	1.1	1.7

The researchers concluded that vinclozolin did not affect pectinase activity.

5. Acid Phophatas Activity

Vinclozolin was added to moist loam soils at 10 mg/kg soil. Samples of 1 g soil were taken at various intervals, and then 2 ml of nitrophenyl-phosphatedisodium salt solution and 2 ml of TRIS-maleate buffer (pH7) were added and incubated 1 hour at 37°C. The reaction was stopped by addeding 5 ml of 0.05N NaOH. The batch was filtered and the filtrate was used for determination of enzyme activity [u/l], using Zeiss photometer. Results are shown in Table 8.

Table 8. Phosphatase Results

ai	Enzyme activity (u/l) after x days			
mg/l	0	7	14	21
0	0.9	1.0	4.2	2.8
10	0.1	1.4	4.6	3.2

The researchers concluded that vinclozolin did not affect pectinase activity.

6. Proteinase Activity

Glucose (0.1%) and albumin (2%) were added to a sterile nutrient solution. Solutions were then inoculated with: Bacillus subtilis, Botrytis cinerea, and Streptomyces aureofaciens. Vinclozolin was then added at concentrations equivlent to 10 mg/l of solution. Samples were taken at various intervals and proteinase activity was determined by measuring albumen concentration, using albumen color reagent. Results are shown in Table 9.

Table 9. Proteinase Results

mg protein/100 nutrient sclution after x days

Organism	ai mg/l	0	4	7	15	21	29
Bacillus	0	1.9	1.6	1.4	0.8	0.4	0.2
subtilis	10	2.0	1.5	1.3	0.7	0.5	0.2
Botrytis	0	1.7	1.1	0.7	0.2	0.2	-
cinerea	10	1.5	1.1	0.7	0.2	0.2	-
Streptomyces	0	2.0	1.9	1.8	1.6	1.4	1.2
aureofaciens	10	2.0	1.9	1.8	1.7	1.4	1.2

The researches concluded that vinclozolin did not affect proteinase activity.

4.0 Summary

4.1 Photolysis: Additional data submitted to those previously reviewed on 3/25/80, showed that vinclozolin is stable to hydrolysis in aqueous solution at pH ≤ 2. Such a solution is not susceptible to unsensitized photolysis by light of wavelength >280 nm.

4.2 Soil Metabolism: Additional data submitted to those previously reviewed on 3/25/80, showed that the ratios of soil bacteria/actinomycetes/fungi population fluctuated widely in 12 U.S. and one German soil. However, microflora populations were found to be independent of soil texture and soil location.

4.3 Effects of Vinclozolin on Microorganisms: Vinclozolin did not affect nitrification or molecular nitrogen fixation where Azotobacter chroococcum or Clostridium Pasteurianum were used as the test organisms (see 12/16/80 review, EFB #648). Additionally, vinclozolin did not effect the enzymatic activities of the enzymes: amylase, cellulase, dehydrogenase, pectinase, acid phosphatase, or proteinase; where Bacillus subtilis, Botrytis cinerea, Strepomyces auerofaciens, Bacillus polymyxa, or Fusarium oxysporum Lycopersici; were used as the source organisms.

5.0 CONCLUSIONS

Environmental chemistry data requirements for elucidating the fate of vinclozolin in the environment havebeen satisfied. Therefore, we concur with the registration of vinclozolin for use on strawberry.

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